

Effect of Ethylene Oxide Fumigation on Amino Acid Composition of Pollen¹

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ABSTRACT

Ethylene oxide fumigation reduced the content of histidine in stored dandelion pollen and of histidine and methionine in rape pollen. *Apis mellifera* L. colonies reared fewer larvae than control colonies when fed fumigated pollen. Ethylene oxide fumigation also destroyed some unidentified lipid attractants contained in pollen. Foraging bees collected less, and bees in the hive consumed less fumigated pollen than unfumigated pollen.

Fumigation with ethylene oxide (ETO) has been used frequently by beekeepers to exterminate pests from combs of honey bees, *Apis mellifera* L., and to sterilize combs which may contain organisms causing disease (Michael 1964). Similarly, pollen harvested in traps placed at the hive entrances may be treated with ETO gas and fed to honey bee colonies during periods of pollen dearth. Recently, however, Herbert and Shimanuki (1971) reported that ETO fumigation caused a reduction of the nutritive value of pollen, and that colonies of bees fed only fumigated pollen were unable to rear larvae. They suggested that ETO fumigation destroyed (*l*)-histidine and (*l*)-methionine, since bees were able to rear some larvae when fed fumigated pollen fortified with the 2 amino acids. In the present investigation, the effect of ETO fumigation on the amino acid composition of bee-collected pollen was determined.

One sample of pollen was from the source used by Herbert and Shimanuki (1971) and was predominantly from dandelion. A 2nd sample was mainly rape pollen from Saskatchewan. The samples were harvested in 1969 and 1971, respectively, and stored in a freezer (−20°C) until used in 1972. ETO fumigation was carried out as described by Herbert and Shimanuki (1971).

For amino acid analysis, samples of the pollens were extracted with pentane to remove fats. Duplicate 20-mg portions were hydrolyzed with 6N HCl in sealed tubes (24 h, 105°C). After filtration and removal of the excess HCl, the hydrolyzed amino acids were analyzed on a Beckman 120C Amino Acid Analyzer.

The pollen samples were assayed for their attractiveness to foraging bees in a flight room (Starrat and Boch 1971). Portions of frozen pollen were ground and mixed with cellulose powder (4.5 g) and pulverized sucrose (0.5 g). This mixture was placed in open petri dishes. In each test, 4 dishes, including controls, were placed on a 1×1-m rotating table (90°/min) and the bees on each dish were counted at 15-sec intervals over a period not exceeding 20 min.

The attractiveness of the pollen samples was tested also on honey bees inside the hive. Pollen was moistened with sufficient sugar syrup to form a solid paste which was spread on the bottom of a 100-mm petri dish. In each test, 4 dishes were inverted at random on the top bars of the combs of a colony in a flight room. The position of the dishes was changed every day, and the time required for the bees to remove the contents of each was recorded.

The ability of the colonies to rear larvae on a diet of fumigated pollen was examined by feeding them cakes of pollen moistened with syrup. Two small colonies were first starved for pollen and placed on empty combs; one colony received a dish with a 10-g cake of fumigated pollen, the other (control) colony received a similar cake made from untreated pollen. The pollen cakes were replaced weekly or when the dishes were empty. A test lasted 4–5 weeks. Five tests were run, each with a new pair of colonies. At the end of each test, the open and capped brood cells were counted.

The amino acid analyses (Table 1) showed that ETO fumigation reduced histidine in both pollen samples. Methionine, on the other hand, was reduced only in the rape pollen. Other amino acids apparently were not affected by the treatment.

Honey bees clearly preferred untreated to fumigated pollen. By varying the amounts of rape and dandelion pollen in the dishes, it was determined that fumigation destroyed ca. 40% and 80%, respectively, of their attractiveness to foraging honey bees and to those inside the hive. Adding ca. 3 mg histidine and 3 mg methionine/g of pollen did not restore full attractiveness. This fact indicates that these amino acids do not function as attractants for honey bees.

Our rearing tests confirmed the observations of Herbert and Shimanuki (1971). No brood was reared to the pupal stage in 3 colonies receiving fumigated dandelion pollen. Brood in all stages was observed in 3 similar colonies receiving untreated dandelion pollen. Some brood was reared in 2 colonies receiving fumigated rape pollen, but the number of pupae was only ca. 25 and 10%, respectively, compared with the brood of 2 colonies receiving untreated rape pollen. In one further test, fumigated dandelion pollen was fed to one colony, while a 2nd received fumigated dandelion pollen that had been fortified with 3 mg each of histidine and methionine/

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Table 1.—Effect of ETO fumigation on amino acid composition^a of dandelion and rape pollens.

Amino acid	Dandelion pollen		Rape pollen	
	Un-treated	ETO-treated	Un-treated	ETO-treated
Lysine	71	65	97	116
Histidine	15	3	34	13
Ammonia	117	135	151	228
Arginine	39	42	61	72
Aspartic Acid	100	96	159	153
Threonine	51	46	93	82
Serine	71	63	120	100
Glutamic Acid	92	88	157	138
Proline	108	89	170	162
Glycine	96	87	144	127
Alanine	92	81	138	128
Half Cystine	12	14	11	12
Valine	59	57	101	101
Methionine	17	16	33	7
Isoleucine	47	44	79	77
Leucine	78	73	128	125
Tyrosine	22	22	43	41
Phenylalanine	36	35	65	62

^a Micromoles per gram of fat-free pollen. Means of 2 determinations.

g cake. Both queens continued to lay eggs, but neither colony reared brood to the pupal stage.

Incomplete restoration of the nutritive value of ETO-fumigated pollen also was reported by Herbert and Shimanuki (1971). Preliminary data from gas-chromatographic analyses of fumigated pollens indicated that ETO treatment also reduced the unsaturated fatty acid content.

Present honey bee diets contain soybean flour and dried brewers yeast, etc., as well as a portion of honey bee-collected pollen to make the diet palatable. Until a completely artificial diet without pollen is developed, nonfumigated pollen should be used together with antibiotics.

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